Title

Parenchymal cells from adult rat liver in nonproliferating monolayer culture. II. Ultrastructural studies.

Journal

The Journal of cell biology, 59(3)

ISSN

0021-9525

Authors

Chapman, GS
Jones, AL
Meyer, UA
et al.

Publication Date

1973-12-01

DOI

10.1083/jcb.59.3.735

Peer reviewed

PARENCHYMAL CELLS FROM ADULT RAT LIVER IN NONPROLIFERATING MONOLAYER CULTURE

II. Ultrastructural Studies

GEOFFREY S. CHAPMAN, ALBERT L. JONES, URS A. MEYER, and D. MONTGOMERY BISSELL

From the Cell Biology Section, Veterans Administration Hospital and the Departments of Medicine and Anatomy, University of California, San Francisco, California 94143

ABSTRACT

Hepatic parenchymal cells from adult rats, established in vitro as a monolayer, have been evaluated by electron microscopy. Within 24 h after the initial seeding, the incubated cells were polygonal and in close apposition with three to six neighboring cells. The ultrastructure of the monolayer cells was examined at this time and after 3 and 10 days of incubation. With the exception of a few enlarged mitochondria, organelles in both the 1- and 3-day monolayer cells were indistinguishable quantitatively and morphologically from those found in the intact liver. After 10 days of incubation, however, the rough-surfaced endoplasmic reticulum (RER) had become dilated and vesiculated. In all cells studied, portions of RER were found in a close spatial relationship to mitochondria. From its frequency, this association appeared to be more than fortuitous, and the organelle complex may represent a functional unit necessary for new membrane formation, as suggested previously. The Golgi complexes of 1- and 3-day cells contained very low density lipoprotein-sized particles, which suggests that the monolayer cells synthesize lipoproteins. These electron microscope observations demonstrate that adult hepatic parenchymal cells in monolayer retain for several days the subcellular structural elements characteristic of normally functioning hepatocytes.

INTRODUCTION

Liver cells respond to the fluctuating requirements of the internal milieu by constantly readjusting various metabolic processes. These metabolic alterations often are associated with morphologic changes. Since Fawcett's (1) classic paper on the fine structure of liver cells, a vast number of investigations have attested to the reproducibility of ultrastructural observations made on the normal liver. Most cell biologists now feel that such studies not only reveal unique, highly detailed structural information, but also provide a reasonably reliable means for evaluating a wide spectrum of hepatocyte functions. For example, the hypertrophy of hepatic smooth endoplasmic reticulum (SER) that occurs in most mammalian liver after phenobarbital administration (2, 3) reflects morphologically the increased ability of the liver to metabolize drugs (2) and steroids (4), to synthesize cholesterol (5), and to conjugate bilirubin (6). Similarly, progesterone can promote

an increase in SER (7), which is paralleled by an increase in microsomal oxidative function (8). Although there are some exceptions (9), the quantity of SER has proven to be a useful criterion for evaluating the activity of enzyme systems associated with these membranes. There is also good agreement between the morphologic organization and secretory activity of liver cells. Production and release of very low density lipoproteins (VLDL) are manifest by the appearance of VLDL within the endoplasmic reticulum (ER) and Golgi complexes (10–12). Furthermore, electron microscopy often detects decreases in metabolic functions. Toxic compounds such as carbon tetrachloride produce decreases in protein synthesis, seen morphologically as polysome dispersion (13). Specific alterations in the mitochondria and other hepatic organelles are characteristic of changes in cellular growth rate (14). Finally, the ultrastructure of the hepatocyte is particularly vulnerable to hypoxia, which causes a series of changes in nuclear chromatin, in the internal architecture of mitochondria, and in the organization of ER (15). In addition to these changes, Glinsmann and Ericsson (16) have found an increase in the number of autophagic vacuoles formed during cell degeneration induced by hypoxia.

A monolayer system for adult rat hepatic parenchymal cells has been described in the preceding paper (17), together with biochemical evidence for the presence of differentiated function in these cells in vitro for several days. The present studies indicate that the same cell preparations possess an essentially normal ultrastructure through at least 3 days of incubation, thus corroborating and extending the biochemical data.

MATERIALS AND METHODS

Cell Isolation and Monolayer Preparation

The details of the technique used in cell isolation and monolayer preparation are covered in the companion article (17) and will not be reiterated here.

Preparation of Monolayer Cells for Electron Microscopy

After incubation periods of 1, 3, and 10 days, dishes of cells were fixed for electron microscopy according to the following method. The monolayer medium was discarded and 4 ml of a mixture containing 2.7% glutaraldehyde and 0.8% paraformaldehyde in 0.08 M sodium cacodylate at pH 7.4 was added to each monolayer-containing Petri dish, covering the intact cell monolayer to a depth of 2 mm. The cells were fixed in the glutaraldehyde-paraformaldehyde mixture for 45 min at room temperature. The monolayer was then washed for 2 h at room temperature with three changes of 0.2 M sodium cacodylate at pH 7.4. Next the cells were postfixed 45 min at room temperature in a final solution of 2% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.4). The monolayer was dehydrated with increasing concentrations of ethanol. En bloc staining was performed during dehydration by immersing the tissue for 30 min in a 2% uranyl acetate solution, using 70% ethanol as the solvent. Final dehydration was in absolute ethanol after which the monolayer, still adherent to the Petri dish, was infiltrated with 50% Epon 812 and 50% absolute ethanol for 30 min before final embedding (18). Polymerization was done in a vacuum oven at 60°C. After polymerization, blocks were thin sectioned in a plane parallel to the monolayer. Sections were stained with alkaline lead citrate by a modification of Reynolds' method (19) and examined in a Philips EM 300 electron microscope.

Cells incubated 1, 3, and 10 days in monolayer were examined as indicated by the following table. Each cell lot was prepared from a different rat.

Incubation time (days)	Number of cell lots	Number of monolayer plates examined
1	2	4
3	3	8
10	1	2

Cells incubated for 3 days were studied in the greatest detail because previous work indicated that monolayers of this age were the most appropriate for biochemical study (17).

Preparation of Regenerated Liver for Electron Microscopy

To provide a comparison to monolayer cells, samples of regenerated liver were prepared as follows: 4 days after a two-thirds hepatectomy, rats were lightly anesthetized with pentobarbital. The portal vein was cannulated with PE-205 tubing and the liver fixed by *in situ* perfusion for 2 min at a rate of 5 ml/min per 100 g of animal body weight according to the method of Wisse (20). The use of an electric infusion pump (model 901, Harvard Apparatus Co., Inc., Millis, Mass.) assured a uniform perfusion rate. Livers were fixed with the glutaraldehyde-paraformaldehyde mixture using either phosphate or cacodylate buffer, with or without preperfusion with

the balanced salt solution used for preparation of isolated hepatic parenchymal cells (17). The phosphate-buffered fixative without preperfusion appeared to give the most consistent results. After perfusion, a distal section of the right lateral lobe of each liver was cut into 1-mm^3 blocks and processed similarly to the monolayer cells.

RESULTS

Regenerated Intact Liver

The fine structure of normal rat liver has been studied extensively and has been described thoroughly in the literature (1, 21). Previous investigations revealed that rat liver cells undergo striking morphological changes during the first day of regeneration after subtotal hepatectomy. As liver regeneration proceeds, hepatocellular ultrastructure is reconstituted so that by the 4th day after hepatectomy most cells exhibit essentially normal cytoplasm (22, 23).

Examining intact liver tissue on day 4 after partial hepatectomy, we found minimal differences between regenerated cells and normal liver parenchymal cells. The bile canaliculi appeared somewhat dilated when compared with those of normal rat liver. Increased numbers of microbodies and lysosomes reported by others were not apparent in our preparations. For ultrastructural studies the regenerated liver was used as a reference point for comparison to cells established in monolayer since the regenerated liver was the tissue from which the monolayer cells were prepared.

Monolayer Cells

As indicated above, the ultrastructure of cells incubated 3 days in monolayer will be described in greatest detail because cells at this stage received the most intensive biochemical study. However, the significant findings in 1-day and 10-day monolayer cells will also be indicated.

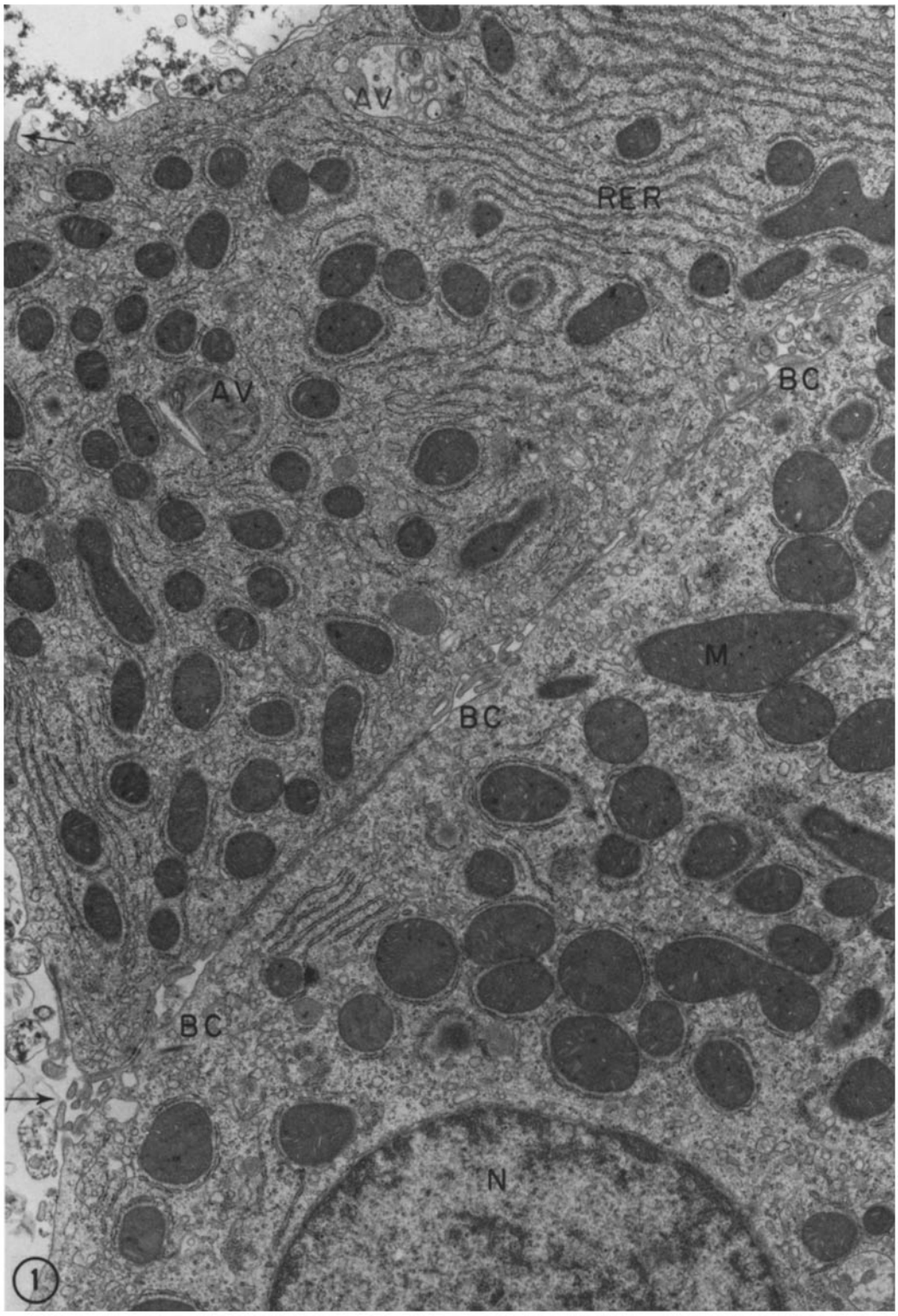
CELL SURFACES: When incubated according to the described technique, parenchymal cells formed a monolayer which adhered to the bottom of a plastic Petri dish. Due to the high density of seeding, the polygonal cells were closely packed after 24 h of incubation, and most cells were in close apposition with three to six neighbors.

Intercellular boundaries fell into two categories. In the first type the two apposing cell membranes were parallel and separated by about 150 Å (Figs. 1 and 2). Occasionally structures quite similar to bile canliculi appeared to interrupt the close apposition of the cell membranes (Fig. 1). Along the second type of intercellular boundary the neighboring cell membranes were separated by an intervening space averaging 1 μm. This latter intercellular space was filled with microvilli formed by plasma membrane evaginations of both apposing cells. These microvilli and those observed along the free surfaces of the monolayer cells were similar to microvilli characteristically found at the sinusoidal surface of normal hepatic parenchymal cells. No clear evidence of junctional complexes was found along the intercellular boundaries. However, structures resembling desmosomes as well as cytoplasmic interdigitations similar to the lateral plication originally described by Fawcett (1) were seen.

NUCLEI: The nuclei of the monolayer cells could not be differentiated from those of the 4-day regenerated liver cells. Chromatin consisted of fine granules scattered throughout the nucleoplasm. Chromatin particles were also aggregated into dense clumps spaced around the nuclear circumference on the inner nuclear membrane (Fig. 1). Ribosomes were found on the cytoplasmic surface of the outer nuclear membrane, nucleoli were often multiple, and binucleate cells were occasionally observed.

ROUGH ENDOPLASMIC RETICULUM (RER): Well-developed lamellar profiles of RER were found in monolayer cells incubated for 1 and 3 days (Figs. 1 and 2). Intact polysome clusters were visible in sections showing *en face* views of RER (Fig. 2). The average quantity of parallel stacks of RER cisternae varied inversely with the length of time the particular cell lot had been maintained in monolayer. They were almost entirely absent after 10 days in monolayer with most of the RER being in the form of discontinous vesicles.

The most striking finding of the RER was that cisternae lay in close association with virtually all mitochrondria of 1-, 3-, and 10-day cells (Figs. 1, 2, 5, and 6). Some mitochondria appeared completely encircled with RER while others were partially surrounded. At some points the perimitochondrial RER lay closer than 100 Å to the outer mitochondrial membrane (Fig. 5). At these points ribosomes were not located on the ER membrane adjacent to the mitochondrion. The perimitochondrial RER in cells incubated


AV
RER
AV
BC
M
BC
BC
N
1

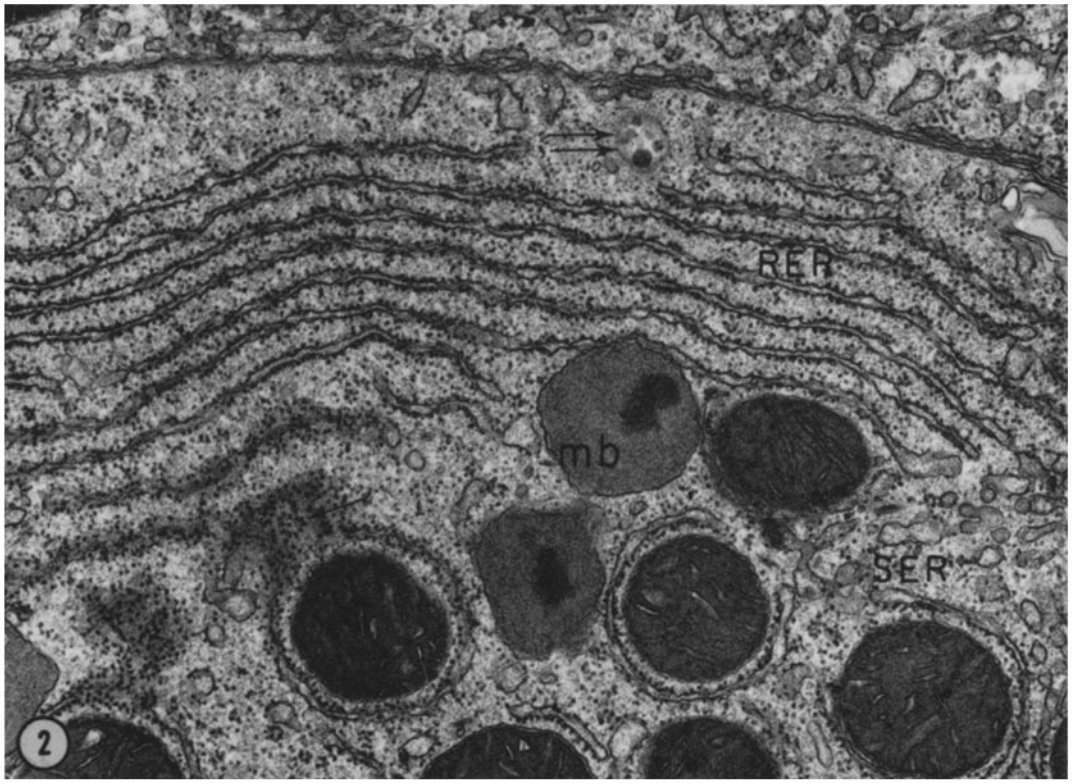


FIGURE 2 1-day cell. An intercellular boundary arches across the upper area of this field. Both SER and RER are present. An *en face* view of RER reveals polysome clusters (arrow). Note the single RER cisternae which partially encircle each mitochondrion. Two microbodies (*mb*) with dark, eccentrically located crystalloids lie near the center of the field. A membrane-bound vesicle containing several lipoprotein-like particles is seen near the intercellular boundary (double arrows). × 30,000.

10 days in monolayer was somewhat dilated and vesiculated (Fig. 6).

SMOOTH ENDOPLASMIC RETICULUM (SER): The SER was well preserved and appeared in about equal quantities in both the 1- and 3-day monolayer cells. Continuities between the SER and RER cisternae were frequent. The SER appeared as interwoven and anastomosing tubules which arborized in small patches throughout the cytoplasm (Fig. 5). These patches never appeared as well developed as they were in regenerated liver or in normal rat liver. The regenerated rat liver

All electron micrographs are of cells grown in monolayer. Age refers to the time of incubation in monolayer.

Abbreviations used in legends:

AV, Autophagic vacuole	*mb*, Microbody
BC, Bile canaliculus	*N*, Nucleus
EM, Enlarged mitochondrion	*RER*, Rough endoplasmic reticulum
M, Mitochondrion	*SER*, Smooth endoplasmic reticulum

FIGURE 1 3-day cells. Portions of two cells are shown. The intercellular boundary, coursing diagonally across the field, is interrupted by structures resembling bile canaliculi (*BC*). A large area of RER is seen in the upper region of this field. Microvilli are found along the free surfaces of the cells and within the bile canaliculus-like structures (arrows). Two large autophagic vacuoles (*AV*) containing various sized vesicles and other debris are seen. Note the large mitochondria (*M*) in the lower cell. × 14,000.

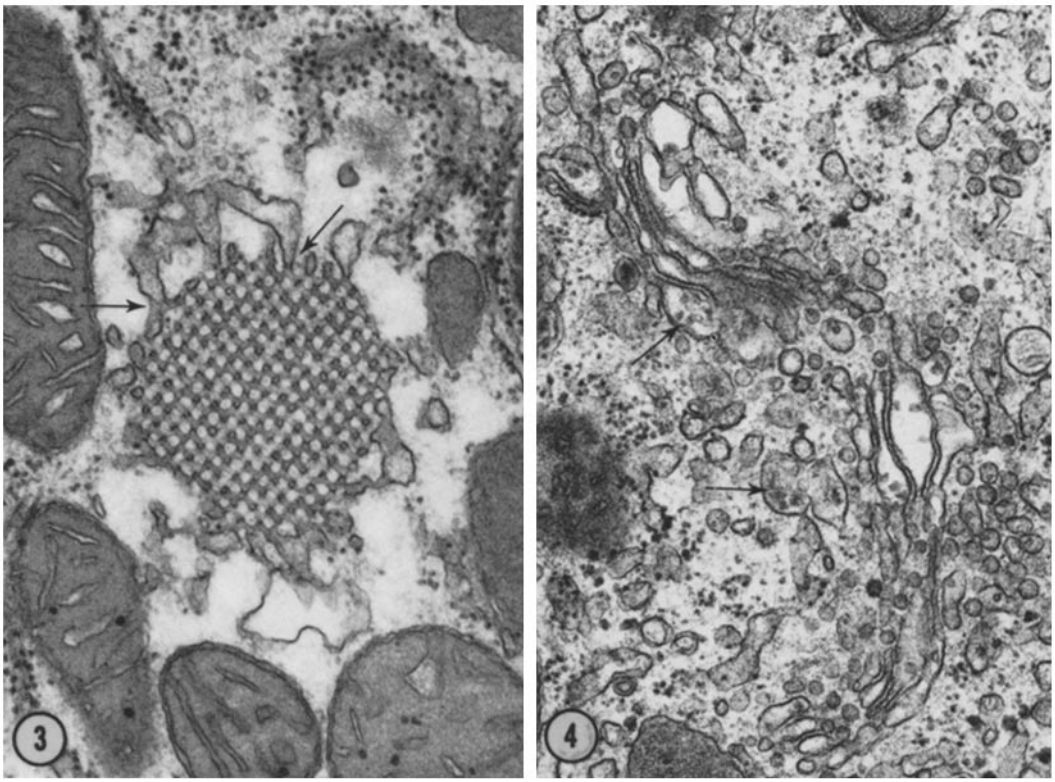


FIGURE 3 1-day cell. An interlacing tubular network is shown consisting of highly ordered repeating units of smooth membrane. Arrows indicate continuity between the lumina of the SER and of the tubular network. × 35,000.

FIGURE 4 3-day cell. Several particles similar in appearance to lipoproteins (arrows) are associated with the Golgi complex. × 38,000.

cells examined after *in situ* fixation of the liver revealed large amounts of intracellular glycogen found in close association with SER. This was not surprising since the rats were fed ad lib. before sacrifice. In contrast, only small pockets of glycogen rosettes were found in cells cultured for 1 and 3 days, with virtually none in 10-day cells (Fig. 6). The latter data are in agreement with chemical measurements of the glycogen content of monolayer cells and reflect the absence of glucose from the incubation medium (17).

In a few sections through the monolayer cells a curious cytoplasmic component was observed. This structure consisted of short tubules delimited by a smooth membrane and oriented parallel to each other, anastomosed in a highly ordered three dimensional network (Fig. 3). The limiting membranes of the tubules forming the network were continuous with the membranes of the SER. These tubular networks in most cases were spherical zones averaging 0.7 μm in diameter.

GOLGI COMPLEXES AND MULTIVESICULAR BODIES: In all the monolayer cells a well-developed Golgi complex was present, but, in contrast to those observed in the intact rat liver, they were not polarized toward the structures resembling bile canaliculi. The Golgi complexes of the monolayer cells (Figs. 4 and 7) consisted of curved, parallel cisternae of smooth membrane often dilated at the terminal portions and surrounded by numerous round vesicles and vacuoles. Osmophilic lipoprotein-like particles were found in almost every Golgi complex of cells after 1 and 3 days in monolayer (Figs. 4 and 7).

Cells examined after 1 and 3 days in monolayer contained spherical bodies bounded by a single smooth membrane with diameters ranging from 0.25 to 0.75 μm. Each body contained from 10 to

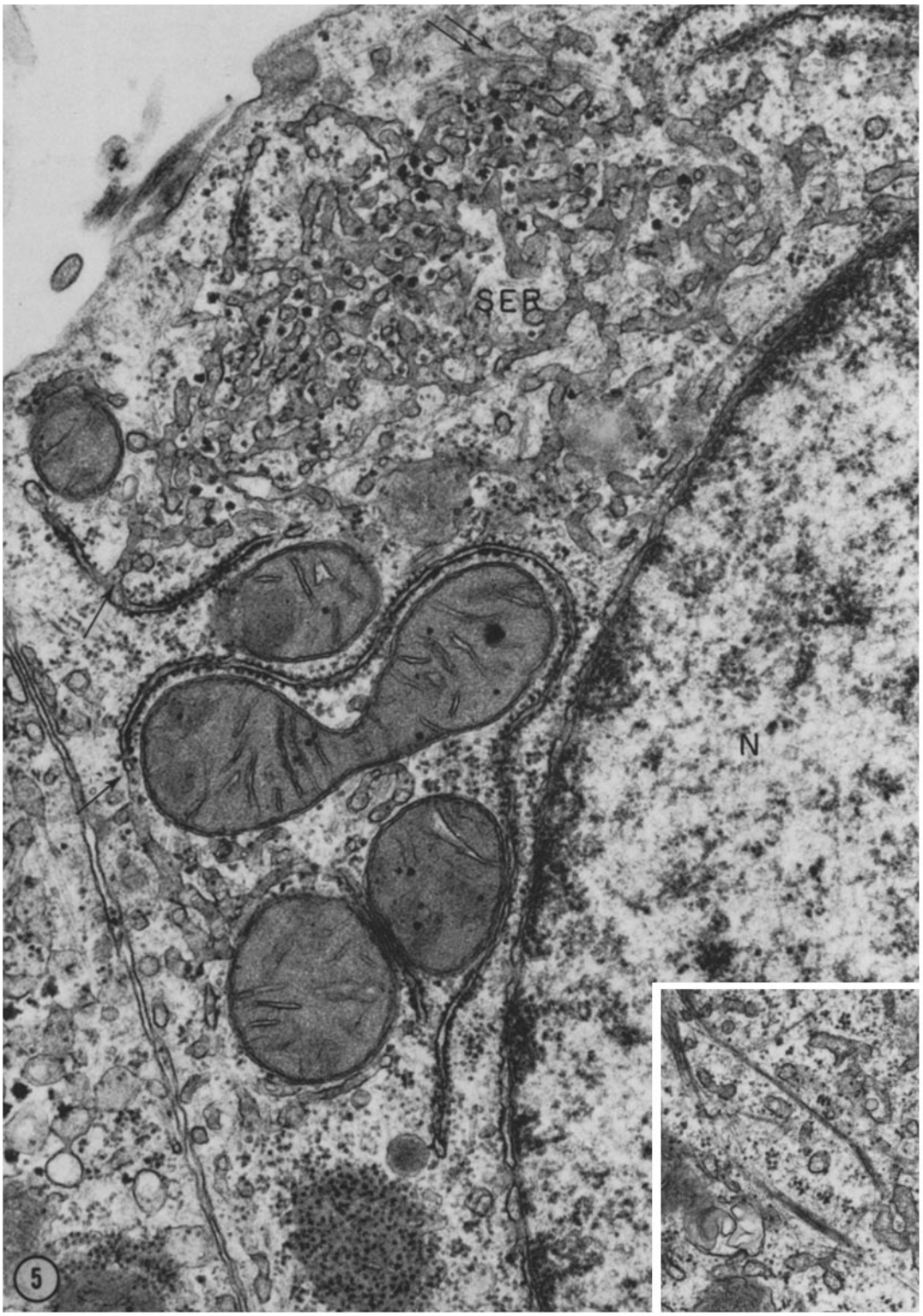


FIGURE 5 1-day cell. An area of SER associated with a few dark-staining glycogen particles lies in the upper part of this electron micrograph. Cisternal profiles of ER appear to partially encircle each mitochondrion. Continuity between the RER and SER is seen (single arrows). Note that in one instance the tubular SER appears to be continuous with RER along the midportion of a cisterna. Double arrows point out microfilament bundles frequently seen near the cell periphery (see *inset*). × 38,000; *inset*, × ~25,000.

FIGURE 6 10-day cells. Generalized dilatation and vesiculation of RER is a characteristic finding in 10-day cells. Many free ribosomes lie in the cytoplasm. In the nucleus (*N*), the chromatin remains well dispersed. × 15,000.

50 spherical droplets of uniform diameter ranging from 400 to 500 Å. These multivesicular bodies were present in all parts of the cytoplasm but were most often found in areas adjoining Golgi complexes.

MITOCHONDRIA AND MICROBODIES: In general the mitochondria of the monolayer cells were normal with respect to membrane integrity, size and form of cristae, appearance of matrix, and size and number of matrix granules. Many cells incubated 3 days in monolayer contained mitochondria larger than are normally found, with lengths up to 4.8 μm. The matrix density, the intramitochondrial granules, and the cristae were well developed and were similar to those in the normal sized mitochondria (Fig. 8). In about 50% of the examined cells, closely packed groups of these enlarged mitochondria were observed (Fig. 8).

Morphologically normal microbodies containing characteristic crystalloids were found with usual frequency in both 1- and 3-day cells (Figs. 2 and 7) but were scarce in 10-day cells.

AUTOPHAGIC VACUOLES: 1-day cells contained many large pleomorphic autophagic vacuoles up to 2.5 μm in diameter (Fig. 7). Most of these contained tortuous concentric whorls of smooth membrane, while others contained recognizable mitochondria and microbodies. Still others appeared empty or contained only amorphous ground substance and a few small round vesicles (Fig. 7). After 3 days in monolayer these large complex structures were not prominent although smaller autophagic vacuoles resembling those found in normal liver were observed.

MICROTUBULES AND MICROFILAMENTS: Microtubules averaging 200 Å in diameter were noted in both regenerated liver cells and monolayer cells at each of the three incubation periods investigated. They were found in all parts of the cytoplasm in random orientation.

In addition, filaments about 60 Å in diameter were found in the monolayer cells with a frequency which increased directly with incubation time (Figs. 5 and 8). They appeared most often near the periphery of 3- and 10-day cells. Occasionally

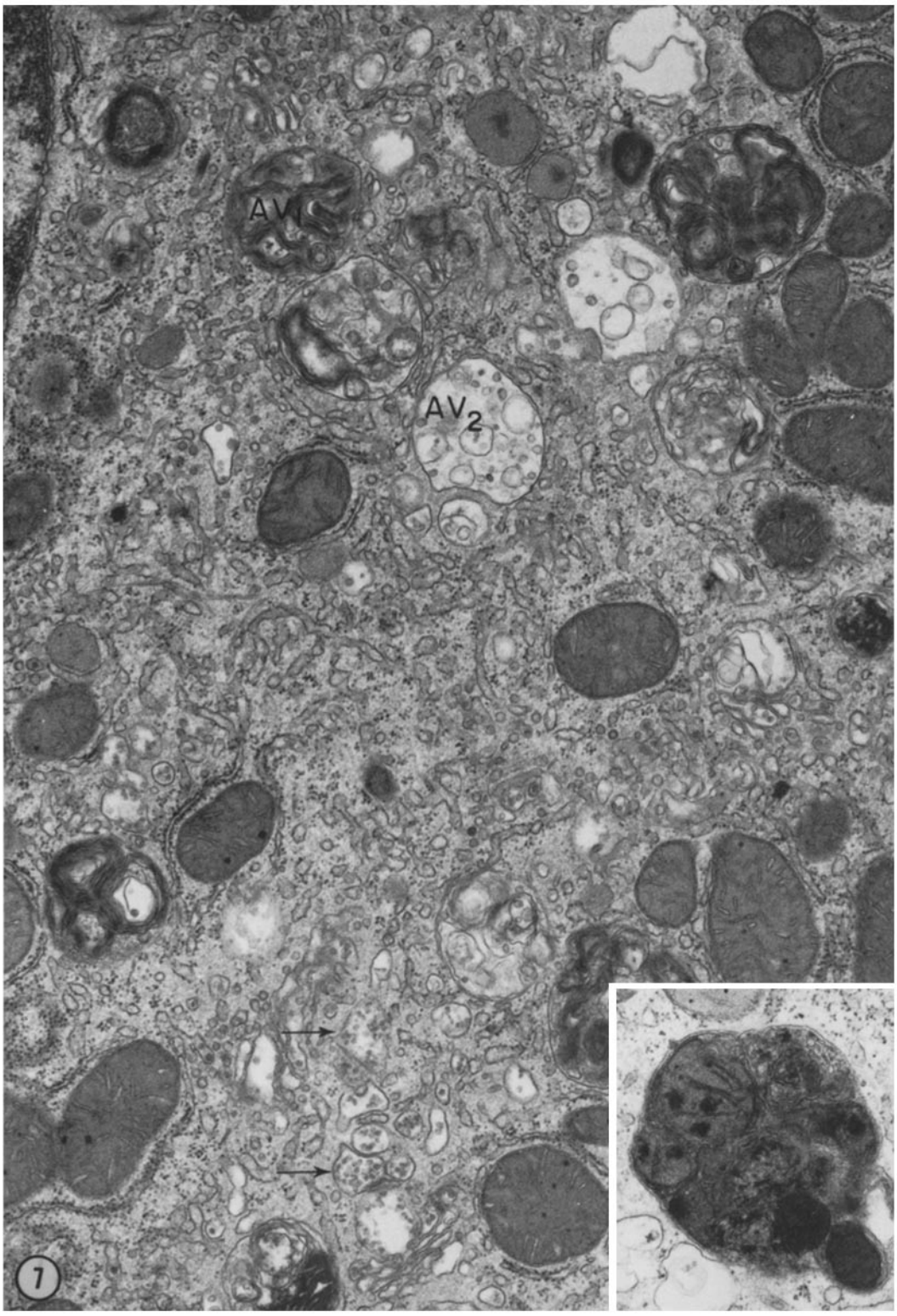


FIGURE 7 1-day cell. Several large autophagic vacuoles at various stages of development dominate the cytoplasm. Some are filled with concentric membranous whorls resembling myelin figures (AV_1), while others contain scattered vesicles of various sizes (AV_2). The *inset* illustrates mitochondria within a large autophagic vacuole. Arrows indicate lipoprotein-like particles in association with the Golgi complex at the bottom of the field. × 22,000; *inset*, × 27,000.

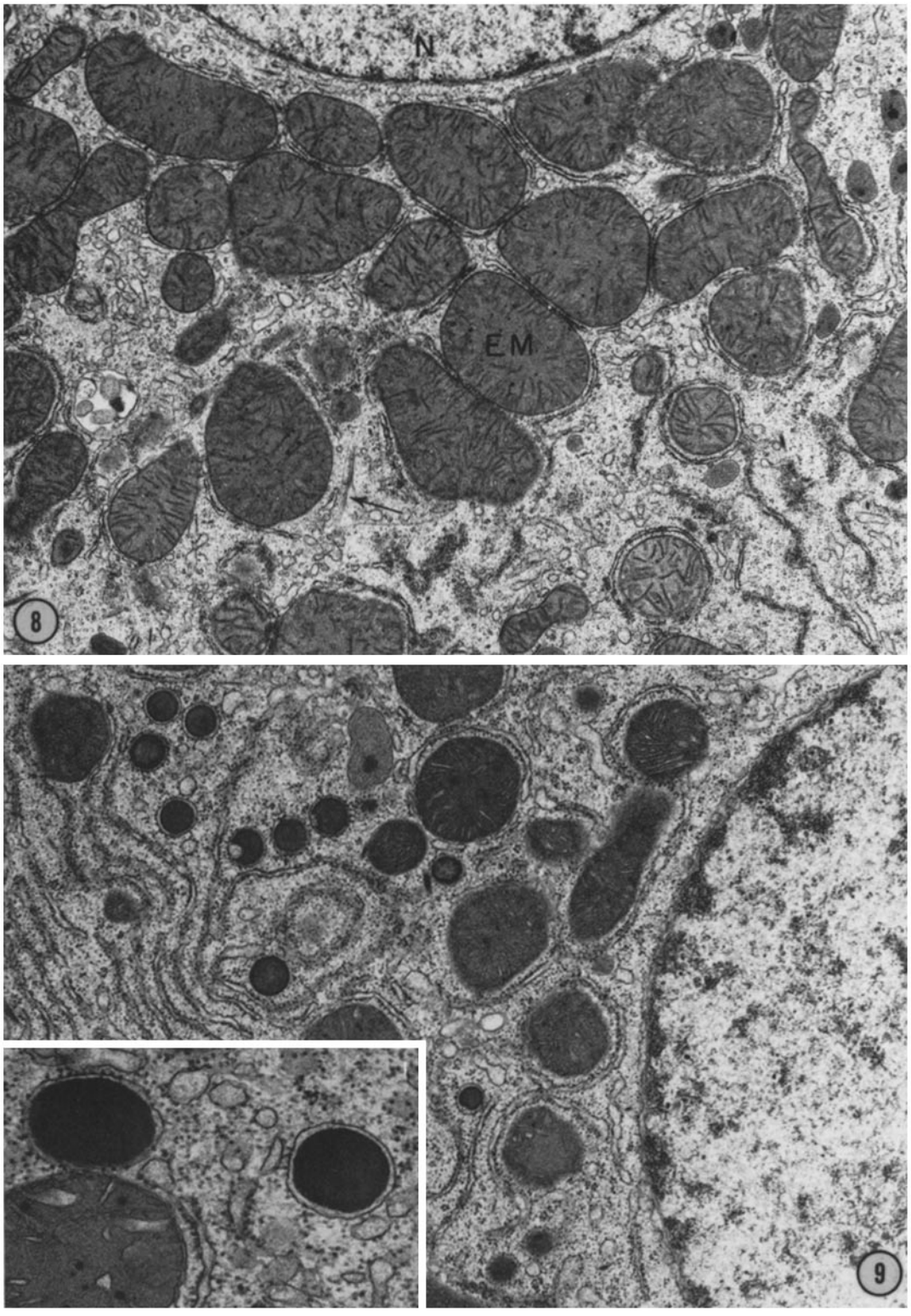


FIGURE 8 3-day cell. Several closely packed enlarged mitochondria (*EM*) are shown. Arrow indicates a bundle of microfilaments. × 13,000.

FIGURE 9 3-day cell. This micrograph illustrates a number of spherical, membrane-bound osmophilic bodies found in a small percentage of monolayer cells. The limiting membranes of these bodies appear to be ribosome studded (*inset*). × 20,000; *inset*, × 33,000.

these filaments were highly organized into bundles (Figs. 5 and 8), some of which were straight and isolated while others curved and anastomosed with neighboring bundles.

LIPID INCLUSIONS: Virtually all regenerated hepatic parenchymal cells contained cytoplasmic lipid droplets without surrounding membranes. Lipid droplets were also visible in 1- and 3-day monolayer cells, but the size and number of droplets per cell was reduced.

In addition, a small but significant number of 3-day cells contained spherical osmophilic particles 0.4 μm in diameter, each of which was encircled by a single, ribosome-studded membrane (Fig. 9). Although present in less than 10% of the 3-day cells, these osmophilic droplets were abundant in those cells where in they were found.

DISCUSSION

These ultrastructural studies document the similarity of hepatic parenchymal cells in monolayer to the intact parent liver and provide a morphologic basis for the presence of differentiated function in this cell system. Cells incubated 1, 3, and 10 days were examined in order to assess the adaptive or degenerative changes that might occur with time in such preparations in vitro. Consistent with the biochemical data reported in the companion paper (17), 3-day cells demonstrated the greatest ultrastructural resemblance to intact liver parenchymal cells.

The salient difference between 1-day and 3-day cells was the presence of large pleomorphic autophagic vacuoles in the cells incubated for 1 day. Studies of rat liver in vivo have suggested that glycogenolysis is a stimulus to autophagic vacuole formation (24). The conditions for the preparation and incubation of hepatocyte monolayers result in glycogenolysis, since the media used contained no glucose (17). Further studies will be required, however, to determine if glycogenolysis and autophagic vacuole formation are causally related in this cell system. Similar structures can result from a variety of cellular insults in vivo, including hypoxia (16) and caloric deprivation (25).

Hepatic parenchymal cells incubated in monolayer for 3 days exhibited well-developed SER and RER which lends morphologic support to the demonstrated ability of these cells to carry out, respectively, *O*-demethylations and synthesis of albumin. Similarly, the Golgi complex was well developed and indistinguishable from that observed in regenerated liver cells. Nearly every Golgi complex observed had large vesicles which contained particles that closely resemble lipoproteins seen in vivo in liver preparations (10–12). This is of particular interest since Berry and Friend (26) noted that cells freshly isolated by similar methodology contain no lipoproteins, an observation confirmed in independent studies by one of the authors (A. L. Jones). Thus, the monolayer cells appear to be capable of *de novo* synthesis of lipoproteins.

The spherical membrane-bound lipoid droplets, found in a small percentage (~10%) of the 3-day monolayer cells, are similar to those produced in liver by orotic acid feeding (27). The lipid accumulation may reflect an inability of the cell to mobilize the particles for secretion, hence they remain within the cisternae of the ER for prolonged periods and accumulate a large fatty core.

The enlarged mitochondria of 3-day cells were normal in every morphological aspect except size, resembling those seen in the liver of partially starved rats (25), which suggests the lack of nutritional elements or energy sources in the monolayer medium. By contrast, deficiencies of iron (28) or essential fatty acids (29), as well as complete starvation (1, 29) and cuprizone feeding (30) result in changes in hepatic mitochondria which are morphologically quite different from those of the monolayer cells.

At all time periods studied, a prominent finding in the monolayer cells was the close apposition of individual mitochondria and segments of RER. The proximity of ER and mitochondria may be important for the transfer of molecules between the two cellular compartments, as in membrane hemoprotein synthesis which requires the export of of heme from mitochondria to newly formed membrane protein (31, 32). The observed frequency of this association in vivo during times of induced membrane growth has previously been discussed (8), including its occurrence during the period immediately before birth.[1]

The generalized disappearance of RER stacks in the 10-day monolayer cells may be due to a deficiency in the monolayer system of a specific factor or factors essential for long-term membrane maintenance. For example, gonadal steroids stimulate SER synthesis in rat liver (7) and maintain RER configuration in adrenalectomized and gonadectomized rats (9). Insulin has also been

[1]A. L. Jones. Unpublished observations.

shown to be necessary for maintenance of RER in intact liver cells (33) and mammary gland cells (34). The monolayer system, which employs a completely defined serum-free medium, lends itself to studies of individual nutritional and hormonal factors such as these which may serve to maintain normal hepatocyte structure and function.

We wish to thank Dr. Elinor S. Mills for a critical reading of the manuscript and Joan Hahn for technical assistance.

This work was supported in part by the Veterans Administration Hospital, San Francisco, Calif., by a National Institutes of Health Medical Student Research Training Fellowship, by United States Public Health Service Special Postdoctoral Fellowship 5-F03-AM 46796 and by United States Public Health Service research grant AM-11275.

Received for publication 8 March 1973, and in revised form 16 July 1973.

REFERENCES

1. FAWCETT, D. W. 1955. Observations on the cytology and electron microscopy of hepatic cells. *J. Natl. Cancer Inst.* **15** (Suppl.):1475.
2. REMMER, H., and H. J. MERKER. 1965. Effects of drugs on the formation of smooth endoplasmic reticulum and drug metabolizing enzymes. *Ann. N. Y. Acad. Sci.* **123**:79.
3. JONES, A. L., and D. W. FAWCETT. 1966. Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital (with a review on the functions of this organelle in liver). *J. Histochem. Cytochem.* **14**:215.
4. CONNEY, A. H., K. SCHNEIDMAN, M. JACOBSON, and R. KUNTZMAN. 1965. Drug induced changes in steroid metabolism. *Ann. N. Y. Acad. Sci.* **123**:98.
5. JONES, A. L., and D. T. ARMSTRONG. 1965. Increased cholesterol biosynthesis following phenobarbital induced hypertrophy of endoplasmic reticulum in liver. *Proc. Soc. Exp. Biol. Med.* **119**:1136.
6. YAFFE, S. J., G. LEVY, T. MATSUZAWA, and T. BALIAH. 1966. Enhancement of glucuronide-conjugating capacity in a hyperbilirubinemic infant due to apparent enzyme induction by phenobarbital. *N. Engl. J. Med.* **275**:1461.
7. EMANS, J. B., and A. L. JONES. 1968. Hypertrophy of liver cell smooth surfaced reticulum following progesterone administration. *J. Histochem. Cytochem.* **16**:561.
8. JONES, A. L., and J. B. EMANS. 1969. The effects of progesterone administration on hepatic endoplasmic reticulum: An electron microscopic and biochemical study. *In* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids. H. A. Salhanick, D. M. Kipnis, and R. L. Vande Wiele, editors. Plenum Press, New York. 68.
9. JONES, A. L., and E. S. MILLS. 1973. Ultrastructural contributions to molecular pharmacology. A guide to Molecular Pharmacology-Toxicology. R. M. Featherstone, editor. Marcel Dekker, Inc., New York.
10. JONES, A. L., N. B. RUDERMAN, and M. G. HERRERA. 1966. An electron microscopic study of lipoprotein production and release by the isolated perfused rat liver. *Proc. Soc. Exp. Biol. Med.* **123**:4.
11. JONES, A. L., N. B. RUDERMAN, and M. G. HERRERA. 1967. Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat liver. *J. Lipid Res.* **8**:429.
12. HAMILTON, R. L., D. M. REGEN, M. E. GRAY, and V. S. LEQUIRE. 1967. I. Electron microscopic identification of very low density lipoproteins in perfused rat liver. *Lab. Invest.* **16**:305.
13. SMUCKLER, E. A., O. A. ISERI, and E. P. BENDITT. 1962. An intracellular defect in protein synthesis introduced by carbon tetrachloride. *J. Exp. Med.* **116**:55.
14. PEDERSON, P. L., J. W. GREENAWALT, T. L. CHAN, and H. P. MORRIS. 1970. A comparison of some ultrastructural and biochemical properties of mitochondria from Morris hepatomes 9618A, 7800 and 3924A. *Cancer Res.* **30**:2620.
15. TRUMP, B. F., P. J. GOLDBLATT, and R. E. STOWELL. 1965. Studies on necrosis of mouse liver in vitro. Ultrastructural alterations in the mitochondria of hepatic parenchymal cells. *Lab. Invest.* **14**:343.
16. GLINSMANN, W. H., and J. L. E. ERICSSON. 1966. Observations on the subcellular organization of hepatic parenchymal cells. II. Evolution of reversible alterations induced by hypoxia. *Lab. Invest.* **15**:762.
17. BISSELL, D. M., L. E. HAMMAKER, and U. A. MEYER. 1973. Parenchymal cells from adult rat liver in nonproliferating monolayer culture. I. Functional studies. *J. Cell Biol.* **59**:722.
18. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.
19. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.
20. WISSE, E. 1970. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J. Ultrastruct. Res.* **31**:125.

21. BRUNI, C., and K. R. PORTER. 1965. The fine structure of the parenchymal cell of the normal rat liver. I. General observations. *Am. J. Pathol.* **46**:691.
22. STENGER, R. J., and D. B. CONFER. 1966. Hepatocellular ultrastructure during liver regeneration after subtotal hepatectomy. *Exp. Mol. Pathol.* **5**:455.
23. VIRÁGH, S., and I. BARTÓK. 1966. An electron microscopic study of the regeneration of the liver following partial hepatectomy. *Am. J. Pathol.* **49**:825.
24. BECKER, F. F. 1972. Acute glycogenolysis: A major stimulus of autophagocytic activity in rat hepatocytes. *Proc. Soc. Exp. Biol. Med.* **140**:1170.
25. HERDSON, P. B., P. J. GARVIN, and R. B. JENNINGS 1964. Fine structural changes produced in rat liver by partial starvation. *Am. J. Pathol.* **45**:157.
26. BERRY, M. N., and D. S. FRIEND. 1969. High-yield preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. *J. Cell Biol.* **43**:506.
27. NOVIKOFF, A. B., P. S. ROHEIM, and N. QUINTANA. 1966. Changes in rat liver cells induced by orotic acid feeding. *Lab. Invest.* **15**:27.
28. DALLMAN, P. R., and J. R. GOODMAN. 1971. The effects of iron deficiency on the hapatocyte. A biochemical and ultrastructural study. *J. Cell Biol.* **48**:79.
29. WILSON, J. W., and E. H. LEDUC. 1963. Mitochondrial changes in the liver of essential fatty acid-deficient mice. *J. Cell Biol.* **16**:281.
30. SUZUKI, K. 1969. Giant hepatic mitochondria: Production in mice fed with cuprizone. *Science (Wash. D. C.).* **163**:81.
31. SCHOLNICK, P. L., L. E. HAMMAKER, and H. S. MARVER. 1969. Soluble hepatic δ-aminolevulinic acid synthetase: End-product inhibition of the partially purified enzyme. *Proc. Natl. Acad. Sci. U. S. A.* **63**:65.
32. MARVER, H. S. 1969. The role of heme in the synthesis and repression of microsomal protein. *In* Microsomes and Drug Oxidations. J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, and G. J. Mannering, editors. Academic Press, Inc., New York. 495.
33. MORGAN, C. R., and R. A. JERSILD, JR. 1970. Alterations in the morphology of rat liver cells influenced by insulin. *Anat. Rec.* **166**:575.
34. MILLS, E. S., and Y. J. TOPPER. 1970. Some ultrastructural effects of insulin, hydrocortisone, and prolactin on mammary gland explants. *J. Cell Biol.* **44**:310.